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Short-range regulatory chromatin loops in plants

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Contents

Summary

I. Introduction

II. Gene looping

III. Intragenic looping

IV. Gene-loops affecting divergent and antisense transcripts

V. Intergenic loops

VI. Enhancer–promoter looping

VII. Defining the edges: small RNAs and DNA methylation in short-range chromatin interactions

VIII. Conclusions

Acknowledgements

References

Summary

In all eukaryotic organisms, gene expression correlates with the condensation state of the chromatin. Highly packed genome regions, known as heterochromatin, are associated with repressed loci, while euchromatic regions represent a relaxed state of the chromatin actively transcribed. However, even in these active regions, associations between chromatin domains dynamically modify genome topology and alter gene expression. Long-range interaction within and between chromosomes determines chromatin domains that help to coordinate transcriptional events. On the other hand, short-range chromatin interactions emerged as dynamic mechanisms regulating the expression of specific loci. Our current capacity to decipher genome topology at high resolution allowed us to identify numerous cases of short range regulatory chromatin interactions that are reviewed in this insight article.

Keywords: Chromatin structure, Small RNAs, DNA methylation, Chromatin loops, Short-range chromatin interactions, Genome topology.

I. Introduction

Eukaryotic cells need to accommodate a large amount of DNA inside their small nuclei. In plants, this can range from the small genome of *Arabidopsis thaliana* (1C=0.16 pg) to the extreme size of *Paris japonica*, which at 1C=152 pg, is 50 times the size of a human genome. To reach such a level of compaction, the genomic DNA adopts organised structures, first by wrapping around nucleosomes and then in higher-level structures of the chromatin (Dogan & Liu, 2018; Stam *et al.*, 2019). The DNA is not arbitrarily distributed in the nucleus, and each chromosome occupies distinctive chromosome territories (CTs) that impact gene accessibility and expression (Gibcus & Dekker, 2013; Liu *et al.*, 2016). In turn, the chromatin can also form topologically associating domains (TADs) that divide large portions of the genome into well-defined, autonomously regulated regions (Dekker *et al.*, 2013; Chung *et al.*, 2016; Liu *et al.*, 2017).

The genome is then organised in chromatin loops, interactions at a local scale that influence gene expression and transcriptional memory (Cavalli & Misteli, 2013; Dekker *et al.*, 2013; Rodriguez-Granados *et al.*, 2016). Chromatin loops are further divided in long-range chromatin interactions, which allow the communication between regions kilobases away in the genome, from the same or different chromosomes (Pontvianne & Liu, 2019), and short-range chromatin interactions (SRCI). SRCIs juxtapose defined regions within a single gene allowing, at a locus scale, very dynamic regulation of gene expression (Rodriguez-Granados *et al.*, 2016). The goal of this article is to review recent discoveries on regulatory SRCIs in plants. We will explore specific cases of functional SRCIs, as well as the elements required for the formation and maintenance of these topological features.

II. Gene-looping

This type of SRCI allows the interactions between the 5' and 3' ends of a gene, "isolating" within a loop an entire transcriptional unit, from the promoter up to the transcription termination site (TTS) (Fig. 1a). The formation of these loops was proposed to enhance gene transcription by allowing more efficient usage of the RNA polymerase II (RNAPII) (Tan-Wong *et al.*, 2008; Larkin *et al.*, 2012; Cavalli & Misteli, 2013). In mammals, for example, the transcription initiation factor TFIIB contributes to the juxtaposition of the promoter and terminator regions at active genes, enabling RNAPII recycling and rapid transcription reinitiation (O'Sullivan *et al.*, 2004; Singh & Hampsey, 2007).

In plants, at least five gene-looping events have been identified (Fig. 1a). Recently, we have identified a case of gene-looping in the sunflower *HaWRKY6* locus that regulates tissue-specifically the expression of the gene (Gagliardi *et al.*, 2019). In cotyledons, a chromatin loop comprising the entire locus, from promoter to TTS, allows efficient recycling of RNAPII increasing *HaWRKY6* levels (Gagliardi *et al.*, 2019). In *Flowering Locus C (FLC),* a loop between the gene promoter and TTS is disrupted after two weeks of cold incubation, leading to a drop in *FLC* expression and flowering transition (Crevillen *et al.*, 2013). Other developmentally-controlled events of gene looping are the cases of *IPT3* and *IPT7*. In both loci, gene-loops promote transcription leading to enhanced cytokinin production, which in turn blocks the progression of the cell cycle in meristematic cells (Jegu *et al.*, 2015). Finally, the *TFL1* locus in Arabidopsis presents a chromatin loop between the promoter and a region located downstream of the TTS that enhances *TFL1* transcription. In the presence of APETALA 1 (AP1), this chromatin loop is disrupted by

allowing the interaction of MADS-box transcription factors to the 3'end region, which results in the repression of the gene (Liu *et al.*, 2013).

III. Intragenic looping

Different from gene looping, where the loops are formed between the gene edges, an intragenic loop only comprises part of a gene. Thus, the portion of the gene included in the loop will largely dictate the regulatory outcome of the chromatin interaction. For example, while a gene-loop formed in the sunflower *HaWRKY6* strongly induces gene expression in cotyledons, a second intragenic loop sharing the same 5'-anchor point, but interacting with the fourth intron of the gene, represses transcription in leaves as RNAPII elongation is blocked (Fig 1b (I), Gagliardi *et al.*, 2019). Similarly, Arabidopsis *FLC* also forms an intragenic loop that negatively regulates the locus. Upon vernalization, and after disruption of the *FLC* gene looping, a *lncRNA* named *COLDWRAP* is transcribed from the *FLC* promoter. Once transcribed, *COLDWRAP* associates with the PRC2 complex inducing the formation of an intragenic loop between the promoter and the first intron of *FLC* that has a repressive effect over the locus (Fig 1b (I), Kim & Sung, 2017). In the *WUSCHEL* (*WUS*) locus, an intragenic loop juxtaposes the flanking regions of the gene and represses its expression. Despite containing the entire coding sequence, this loop excludes the promoter region, which presumably impairs gene expression by blocking the recruitment of RNAPII (Fig 1b (II), Guo *et al.*, 2018).

Even when still not described in plants, intragenic loops can induce the alternative splicing of exons. For example, the CCCTC-binding factor mediates the formation of chromatin loops between promoters and intragenic regions, promoting exon inclusion (Fig 1b (III), Ruiz-Velasco *et al.*, 2017). While still hypothetical, we also envision that intragenic loops could lead to blockage of the full-length transcription, promoting the alternative usage of cryptic polyadenylation sites, resulting in shorter mRNAs (Fig. 1b (IV)).

IV. Gene-loops affecting divergent and antisense transcripts

Upon binding to a promoter, the RNAPII can initiate transcription in both directions producing two different RNA molecules. This phenomenon, known as divergent transcription, is common in most active promoters in eukaryotic cells (Seila *et al.*, 2009). In *S. cerevisiae,* a gene-loop event enhances the directionality of the *FMP27* promoter by impairing the transcription of a divergent *ncRNA* (Fig. 1c (I)). Presumably, this occurs as the RNAPII activity is restricted within the loop,

excluding transcriptional units outside it. In concordance, a mutation in *Ssu72,* an RNA polymerase II CTD phosphatase component of the Cleavage and Polyadenylation Factor that disrupts intragenic looping, leads to a genome-wide increment of divergent transcription (Tan-Wong *et al.*, 2012). In plants, the gene-loop enhancing *HaWRKY6* transcription also forces unidirectional transcription of its promoter, promoting the transcription of the coding sequence but blocking the divergent transcription of a *ncRNA* (Fig. 1c (I), Gagliardi *et al.*, 2019). Such reduction of the divergent transcription impacts the locus dynamics, as the repressed *ncRNA* is required for the establishment of the loop. In this way, the locus reaches a self-buffered equilibrium where the formation of the loop enhances gene transcription but represses the *ncRNA,* progressively promoting the disruption of the SRCI.

Divergent transcription differs from antisense transcription where two promoter regions, flanking a coding sequence, allow the transcription of both strands of a single gene. SRCIs also affect antisense transcription of complementary RNAs, for example in the *FLC* locus. This locus encodes three *ncRNAs*: *COLDWRAP* that derives from the promoter of *FLC* (Kim & Sung, 2017); *COLDAIR* that is transcribed in sense orientation from the first intron of the gene (Heo & Sung, 2011; Crevillen *et al.*, 2013); and *COOLAIR,* which is transcribed in antisense to *FLC* and requires a dedicated promoter located downstream of the TSS of *FLC*. The *COOLAIR* regulatory region resides precisely where the 3' anchor point of the *FLC* gene-loop was predicted (Crevillen *et al.*, 2013). Even when the precise influence of the *FLC*-loop over *COOLAIR* transcription needs to be addressed, a perfect correlation between the loop disruption and an increment in *COOLAIR* transcription was observed (Crevillen *et al.*, 2013; Whittaker & Dean, 2017). This suggests that the *FLC*-loop impairs antisense transcription of *COOLAIR,* probably by blocking RNAPII progression (Fig. 1c (II)).

V. Intergenic loops

This type of SRCI defines those interactions that involve the juxtaposition of chromatin regions between adjacent genes. Given the dense gene distribution of some plant genomes, intergenic loops are potentially frequent. Intergenic loops may act by bringing together regulatory elements of adjacent genes but could also comprise the entire promoter region of divergent loci restricting the transcription of both RNAs simultaneously. Perhaps the most prominent example occurs between the *PID* and *APOLO* loci in Arabidopsis plants. *APOLO* is an auxin-induced *lncRNA* located 4900 bp upstream of the *PID* transcription start site in a divergent orientation. In the

absence of auxin, a chromatin loop encompassing the *PID*/*APOLO* intergenic region and including both promoters is formed repressing both genes transcription (Fig. 1d). Upon auxin signalling, the *APOLO* locus is demethylated, and the loop disrupted, which in turn promotes the divergent transcription of *PID* and *APOLO*. Progressively, the accumulation of *APOLO ncRNA* triggers remethylation of the locus, and re-establishment of the repressive loop (Ariel *et al.*, 2014).

VI. Enhancer–promoter looping

This type of SRCIs brings together distal enhancer elements to the promoters of genes (Fig. 1e, Krivega & Dean, 2012). The first example of enhancer–promoter looping in plants was reported for the *b1* locus of *Zea mays*. Two epialleles, B-I and B', control the expression of this locus by forming chromatin multiloops between the *b1* TSS and upstream sequences. In B-I, an enhancer located 100 kb upstream has an open chromatin state that allows the formation of a multiloop structure that enhances transcription of b1. In the B' epiallele, the enhancer has compact chromatin that reduces the formation of the multiloop and represses *b1* expression (Louwers *et al.*, 2009).

Two chromatin loops located upstream the *FLOWERING LOCUS T* (*FT*) TSS allow the juxtaposition of CORE elements, bounded by CONTANS, and CCAAT boxes, bound by NF-Y (Cao *et al.*, 2014). The formation of this SRCI allows the recruitment of CONTANS to the *FT* promoter, enhancing its transcription (Fig. 1e). Similarly, a SRCI is formed over the *VERDANDI (VDD)* promoter by the heterodimerisation of SEEDSTICK and SEPALLATA3 enhancing gene expression and ovule development (Mendes *et al.*, 2013).

VII. Defining the edges: small RNAs and DNA methylation in short-range chromatin interactions

Currently, the best understood cases of how loops are formed are those involving protein-protein interaction between transcription factors, such as the loops at *WUS*, *VDD,* and *FT* loci (Mendes *et al.*, 2013; Cao *et al.*, 2014; Guo *et al.*, 2018). For example, the transcription factors AGAMOUS (AG) and TERMINAL FLOWER 2 (TFL2) interact with the TSS and TTS flanking regions of *WUS* and allows, upon the interaction between them, the formation of a loop that represses gene expression during flower development (Guo *et al.*, 2018).

For most other cases, histone modifications appeared to lead the establishment of chromatin loops. One of the best-understood examples corresponds to the loop between the *PID* and *APOLO* loci.

The formation of this loop relies on *APOLO*-triggered deposition of H3K27me3 repressive marks across the *PID/APOLO* intergenic region. In turn, H3K27me3 marks serve as anchor points to recruit LHP1 that interact with *APOLO* bridging the methylated histones and establishing the intergenic loop (Ariel *et al.*, 2014).

In *FLC*, the histone-remodelling complex SWI/SNF, particularly its BAF60 subunit, associates with the locus, increasing H3K27me3 marks and reducing H3K9Ac. These modifications lead to the disruption of the *FLC* gene-loop repressing gene expression (Jegu *et al.*, 2014). BAF60 also binds to the promoter regions of *IPT3*, *IPT7*, and *KRP7* during root development. BAF60 reduces H3K4me3 marks and RNAPII recruitment, and increases H3K27me3 deposition destabilising the gene-loops on these loci with the concomitant repression of the genes (Jegu *et al.*, 2015).

The question that arises is how the loop's anchor points are defined. The answer seems to involve DNA methylation of specific regions that precede the histone modifications and loop formation. This is the case of the *PID/APOLO* loci, where RNA-directed DNA methylation (RdDM)-mediated *de-novo* methylation of specific regions across the *APOLO* gene body stabilises the loop formation. The fact that RdDM can trigger SRCIs, points to small RNAs as early signals in the process. Coincidently, abundant small RNAs map the 3'-end region of the *FLC* locus where its gene-loop anchor point resides (Swiezewski *et al.*, 2007; Crevillen *et al.*, 2013). Recently, we have also shown that the formation and maintenance of loops detected over the sunflower *HaWRK6* gene depend on the RdDM pathway and 24 nt small RNAs to define the SRCI anchor points. Furthermore, variations in the levels of small RNAs mapping a region downstream the TTS dictates whether a gene-loop or intragenic-loop is formed over the locus (Gagliardi *et al.*, 2019).

How these small RNAs are generated in the first place is another interesting question. In the case of *PID/APOLO,* the DNA methylation of the locus appears to depend on the levels of the *APOLO* transcript. Thus, it could be expected that the small RNAs are produced from this *ncRNA* and act in *cis*. Remarkably, *APOLO*, can also control loop formation in *trans* by generating DNA-RNA duplexes, thought sequence complementarity, with the anchor points of several SRCIs (Ariel *et al.*, 2020). In *FLC,* small RNAs map a region where no transcript is expected, suggesting that these small RNAs are produced in *trans* (Swiezewski *et al.*, 2007; Crevillen *et al.*, 2013). However, it is possible that double-stranded RNAs, formed by the hybridisation of the complementary mRNAs of *FLC* and *COOLAIR*, serve as a template for siRNA production. In the *HaWRKY6* locus, the small RNAs targeting both 3' anchor points are likely produced in *trans*.

Conversely, the 5'-anchor point is methylated by small RNAs produced by an *ncRNA* transcribed divergently from the coding gene (Gagliardi *et al.*, 2019).

Methylated regions located near genes are commonly associated with transcriptional repression. However, the H3K9me2 methyltransferases SU(VAR)3-9 homologs, SUVH1 and SUVH3, were found to associate DNAJ domain-containing proteins and to bind RdDM-methylated DNA. The recruitment of the DNAJ proteins to these regions enhances the expression proximal genes showing that DNA methylation can also induce expression (Harris *et al.*, 2018). This report opens the door to speculate whether the SUVH1/SUVH3 - DNAJ1/2 complex directly enhances transcription, or if it serves as an anchor point for the formation of positive *SRCI* in the loci, explaining the observed enhanced transcription.

VIII. Conclusions

Chromatin interactions at single locus scale are emerging as particularly dynamic elements of gene regulation (Rodriguez-Granados *et al.*, 2016). The precise mechanisms by which SRCIs control gene expression is not always clear. In most cases, it appears to involve a physical restriction either by clustering regulatory elements to facilitate transcription (Crevillen et al 2013, Jegu et al 2014), or by hampering RNAPII activity (Tan-Wong et al 2012). Many of the chromatin interactions currently reported have been identified in *Arabidopsis thaliana.* Loops affecting divergent genes, as well as intergenic loops, are more likely to occur in Arabidopsis than in other plants given its compact genome size. On the other hand, plants with larger genomes are richer in repetitive elements, such as MITEs, (Lu *et al.*, 2012) that may have a deep impact on the chromatin topology and nearby gene expression as shown for the *HaWRKY6* locus (Gagliardi *et al.*, 2019). Thus, it can be expected that some features of the Arabidopsis chromatin topology are not conserved in other plants. Another aspect that require further investigation is how SRCIs are formed and maintained in plants as it is unclear which factors are required to promote loop formation and how these interactions are stabilised them over time.

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References

Ansari A, Hampsey M. 2005. A role for the CPF 3'-end processing machinery in RNAP II-dependent gene looping. *Genes Dev* **19**(24): 2969-2978.

Ariel F, Jegu T, Latrasse D, Romero-Barrios N, Christ A, Benhamed M, Crespi M. 2014. Noncoding transcription by alternative RNA polymerases dynamically regulates an auxin-driven chromatin loop. *Mol Cell* **55**(3): 383-396.

Ariel F, Lucero L, Christ A, Mammarella MF, Jegu T, Veluchamy A, Mariappan K, Latrasse D, Blein T, Liu C, et al. 2020. R-Loop Mediated trans Action of the APOLO Long Noncoding RNA. *Molecular Cell*. **77(**5): 1055-1065.

Cao S, Kumimoto RW, Gnesutta N, Calogero AM, Mantovani R, Holt BF, 3rd. 2014. A distal CCAAT/NUCLEAR FACTOR Y complex promotes chromatin looping at the FLOWERING LOCUS T promoter and regulates the timing of flowering in Arabidopsis. *Plant Cell* **26**(3): 1009-1017.

Cavalli G, Misteli T. 2013. Functional implications of genome topology. *Nat Struct Mol Biol* **20**(3): 290-299.

Chung IM, Ketharnathan S, Kim SH, Thiruvengadam M, Rani MK, Rajakumar G. 2016. Making Sense of the Tangle: Insights into Chromatin Folding and Gene Regulation. *Genes (Basel)* **7**(10): 71.

Crevillen P, Sonmez C, Wu Z, Dean C. 2013. A gene loop containing the floral repressor FLC is disrupted in the early phase of vernalization. *EMBO J* **32**(1): 140-148.

Dekker J, Marti-Renom MA, Mirny LA. 2013. Exploring the three-dimensional organization of genomes: interpreting chromatin interaction data. *Nat Rev Genet* **14**(6): 390-403.

Dogan ES, Liu C. 2018. Three-dimensional chromatin packing and positioning of plant genomes. *Nat Plants* **4**(8): 521-529.

Gagliardi D, Cambiagno DA, Arce AL, Tomassi AH, Giacomelli JI, Ariel FD, Manavella PA. 2019. Dynamic regulation of chromatin topology and transcription by inverted repeat-derived small RNAs in sunflower. *Proc Natl Acad Sci U S A* **116**(35): 17578-17583.

Gibcus JH, Dekker J. 2013. The hierarchy of the 3D genome. *Mol Cell* **49**(5): 773-782.

Guo L, Cao X, Liu Y, Li J, Li Y, Li D, Zhang K, Gao C, Dong A, Liu X. 2018. A chromatin loop represses WUSCHEL expression in Arabidopsis. *Plant J* **94**(6): 1083-1097.

Harris CJ, Scheibe M, Wongpalee SP, Liu W, Cornett EM, Vaughan RM, Li X, Chen W, Xue Y, Zhong Z, et al. 2018. A DNA methylation reader complex that enhances gene transcription. *Science* **362**(6419): 1182-1186.

Heo JB, Sung S. 2011. Vernalization-mediated epigenetic silencing by a long intronic noncoding RNA. *Science* **331**(6013): 76-79.

Jegu T, Domenichini S, Blein T, Ariel F, Christ A, Kim SK, Crespi M, Boutet-Mercey S, Mouille G, Bourge M, et al. 2015. A SWI/SNF Chromatin Remodelling Protein Controls Cytokinin Production through the Regulation of Chromatin Architecture. *PLoS One* **10**(10): e0138276.

Jegu T, Latrasse D, Delarue M, Hirt H, Domenichini S, Ariel F, Crespi M, Bergounioux C, Raynaud C, Benhamed M. 2014. The BAF60 subunit of the SWI/SNF chromatin-remodeling complex directly controls the formation of a gene loop at FLOWERING LOCUS C in Arabidopsis. *Plant Cell* **26**(2): 538-551.

Kim DH, Sung S. 2017. Vernalization-Triggered Intragenic Chromatin Loop Formation by Long Noncoding RNAs. *Dev Cell* **40**(3): 302-312 e304.

Krivega I, Dean A. 2012. Enhancer and promoter interactions-long distance calls. *Curr Opin Genet Dev* **22**(2): 79-85.

Larkin JD, Cook PR, Papantonis A. 2012. Dynamic reconfiguration of long human genes during one transcription cycle. *Mol Cell Biol* **32**(14): 2738-2747.

Liu C, Cheng YJ, Wang JW, Weigel D. 2017. Prominent topologically associated domains differentiate global chromatin packing in rice from Arabidopsis. *Nat Plants* **3**(9): 742-748.

Liu C, Teo ZW, Bi Y, Song S, Xi W, Yang X, Yin Z, Yu H. 2013. A conserved genetic pathway determines inflorescence architecture in Arabidopsis and rice. *Dev Cell* **24**(6): 612-622.

Liu C, Wang C, Wang G, Becker C, Zaidem M, Weigel D. 2016. Genome-wide analysis of chromatin packing in *Arabidopsis thaliana* at single-gene resolution. *Genome Res* **26**(8): 1057-1068.

Louwers M, Bader R, Haring M, van Driel R, de Laat W, Stam M. 2009. Tissue- and expression level-specific chromatin looping at maize b1 epialleles. *Plant Cell* **21**(3): 832-842.

Lu C, Chen J, Zhang Y, Hu Q, Su W, Kuang H. 2012. Miniature inverted-repeat transposable elements (MITEs) have been accumulated through amplification bursts and play important roles in gene expression and species diversity in *Oryza sativa*. *Mol Biol Evol* **29**(3): 1005-1017.

Mendes MA, Guerra RF, Berns MC, Manzo C, Masiero S, Finzi L, Kater MM, Colombo L. 2013. MADS domain transcription factors mediate short-range DNA looping that is essential for target gene expression in Arabidopsis. *Plant Cell* **25**(7): 2560-2572.

O'Sullivan JM, Tan-Wong SM, Morillon A, Lee B, Coles J, Mellor J, Proudfoot NJ. 2004. Gene loops juxtapose promoters and terminators in yeast. *Nat Genet* **36**(9): 1014-1018.

Pontvianne F, Liu C. 2019. Chromatin domains in space and their functional implications. *Curr Opin Plant Biol* **54**: 1-10.

Rodriguez-Granados NY, Ramirez-Prado JS, Veluchamy A, Latrasse D, Raynaud C, Crespi M, Ariel F, Benhamed M. 2016. Put your 3D glasses on: plant chromatin is on show. *J Exp Bot* **67**(11): 3205-3221.

Ruiz-Velasco M, Kumar M, Lai MC, Bhat P, Solis-Pinson AB, Reyes A, Kleinsorg S, Noh KM, Gibson TJ, Zaugg JB. 2017. CTCF-Mediated Chromatin Loops between Promoter and Gene Body Regulate Alternative Splicing across Individuals. *Cell Syst* **5**(6): 628-637 e626.

Seila AC, Core LJ, Lis JT, Sharp PA. 2009. Divergent transcription: a new feature of active promoters. *Cell Cycle* **8**(16): 2557-2564.

Singh BN, Hampsey M. 2007. A transcription-independent role for TFIIB in gene looping. *Mol Cell* **27**(5): 806-816.

Stam M, Tark-Dame M, Fransz P. 2019. 3D genome organization: a role for phase separation and loop extrusion? *Curr Opin Plant Biol* **48**: 36-46.

Swiezewski S, Crevillen P, Liu F, Ecker JR, Jerzmanowski A, Dean C. 2007. Small RNA-mediated chromatin silencing directed to the 3' region of the Arabidopsis gene encoding the developmental regulator, FLC. *Proc Natl Acad Sci U S A* **104**(9): 3633-3638.

Tan-Wong SM, French JD, Proudfoot NJ, Brown MA. 2008. Dynamic interactions between the promoter and terminator regions of the mammalian BRCA1 gene. *Proc Natl Acad Sci U S A* **105**(13): 5160-5165.

Tan-Wong SM, Zaugg JB, Camblong J, Xu Z, Zhang DW, Mischo HE, Ansari AZ, Luscombe NM, Steinmetz LM, Proudfoot NJ. 2012. Gene loops enhance transcriptional directionality. *Science* **338**(6107): 671-675.

Whittaker C, Dean C. 2017. The FLC Locus: A Platform for Discoveries in Epigenetics and Adaptation. *Annu Rev Cell Dev Biol* **33**: 555-575.

Figure 1: Short-range chromatin loops at individual loci. Types and known, or hypothetical, examples of chromatin loops juxtaposing closely located regions within single loci in plants. **(a)** Gene looping, an interaction between the 5' and 3' ends of a gene that commonly promotes transcription. **(b)** Different types of intragenic loops where interactions occurs within different parts of a gene. (**I)** Interactions comprising the promoter and a part of a gene, with a repressive effect over transcription. (**II)** Interactions excluding the promoter that result in a reduction of the gene transcription rate Interactions between promoters and intragenic regions that lead to alternative splicing events (**III**), or alternative usage of cryptic polyadenylation sites (**IV**). **(c)** Gene-loops affecting divergent (**I**) and antisense (**II**) transcription. **(d)** Intergenic looping, interactions involving the juxtaposition of chromatin regions between adjacent genes. **(e)** Enhancer-promoter looping, structures which bring together distal enhancer elements to the promoters of genes. Known Examples (KE), Regulatory Effect (RE), and Potential Regulatory Mechanism (PRM) are noted on the right side of a graphical representation of different types of loops.

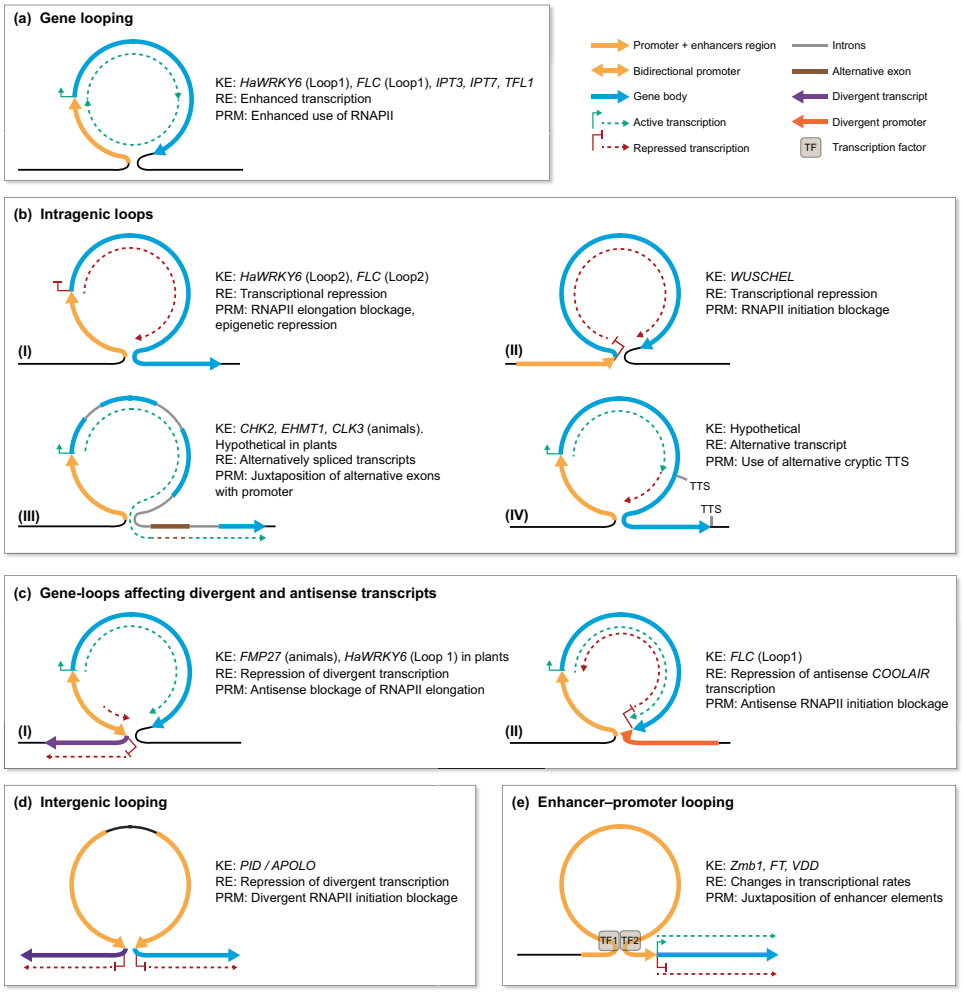

Figure 1

Tansley Insight 32500